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Comparative investigation of the binding characteristics of poly-L-lysine and chitosan on alginate hydrogel

Ying Ren[a,c], Hongguo Xie[a,*], Xiaocen Liu[a,c], Jie Bao[b], Weiting Yu[a,*], Xiaojun Ma[a]

[a] Laboratory of Biomedical Material Engineering, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China
[b] State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, China
[c] University of Chinese Academy of Sciences, Beijing 100049, China

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ABSTRACT

The binding properties of poly-L-lysine and chitosan to alginate have been evaluated quantitatively and compared. Poly-L-lysine bound to alginate hydrogel more rapidly than chitosan as poly-L-lysine has a smaller molar hydrodynamic volume. In addition, poly-L-lysine showed a much higher binding capacity (6.14:1) for alginate hydrogel beads than chitosan (2.71:1), and a little higher binding stoichiometry (0.58) to sodium alginate molecules in solution than chitosan (0.49). An exothermic heat of alginate-poly-L-lysine complexes formation of 2.02 kJ/mol was detected. For alginate–chitosan complexes, the binding enthalpy has been seen to be −3.49 kJ/mol. The stability of the polyelectrolyte complexes was related to their binding enthalpy. The alginate-poly-L-lysine complexes could be disintegrated and rebuilt. By contrast, chitosan was bound with alginate in a steady state. These results provide fundamental insights regarding the structure and property relationships of macromolecules, and will be helpful in designing and selecting appropriate polymers.

1. Introduction

Microcapsules with a hydrogel core and a polyanion–polycation membrane have been proved to possess various applications in biomedical and pharmaceutical areas [1–3]. Although many polymers have been attempted, alginate-poly-L-lysine and alginate–chitosan microcapsules are still the commonly used microcapsule systems [4,5]. Alginate is composed of linear chains of α-L-guluronic acid and β-D-mannuronic acid residues and bound through 1, 4-glycosidic linkages [6,7]. Poly-L-lysine is a peptide having excellent structural precision in terms of molecular weight and secondary structure elements [8]. Chitosan is a linear copolymer polysaccharide, which is made up of various amounts of β (1-4)-linked 2-amino-2-deoxy-D-glucose (D-glucosamine) and 2-acetamido-2-deoxy-D-glucose (N-acetyl-D-glucosamine) units [3,9]. The carboxylic acid groups on alginate make it can crosslink with multiple divalent cations and thus form hydrogels [10]. Amino groups on polycations can interact with alginate hydrogel to form microcapsule membrane on the surface of the hydrogel.

The microcapsule membrane has been demonstrated to be of great importance not only for transport properties (semipermeable membrane) but as well for stability. Previous studies indicated that microcapsules made of poly-L-lysine and chitosan showed different swelling behavior and mechanical properties [11,12]. The stability of alginate-poly-L-lysine microcapsules in physiological conditions was reduced due to the swelling behavior. Alginate-poly-L-lysine microcapsules were ruptured in shaking flask. This limited stability of microcapsules hampered their application [13]. As the polyanion–polycation membrane is formed by the reaction between carboxylate moieties on alginate and protonated amines on chitosan or poly-L-lysine, it is believed that the physicochemical properties of polyanion–polycation membrane could be tailored by controlling the degree of association between the functional groups [14–16]. Also, as the membrane is formed by binding of polycations on calcium alginate hydrogel beads, control of the molecules at this level requires a comprehensive understanding of the binding properties of this system.

The differences in molecular structure will affect their binding with alginate. It was reported that there were different interaction mechanisms between chitosan–alginate and poly-L-lysine–alginate during the assembly process [17]. Poly-L-lysine had the ability to diffuse “in” and “out” of the alginate-based polyelectrolyte films while chitosan was typically confined to the films [18,19], which suggested that the binding affinity of alginate was much stronger with chitosan than poly-L-lysine. However, this

* Corresponding authors at: Laboratory of Biomedical Materials Engineering, Dalian Institute of Chemical Physics (DICP), Chinese Academy of Sciences (CAS), 457 Zhongshan Road, Dalian 116023, PR China.
E-mail addresses: xiehg@dicp.ac.cn (H. Xie), yuwt@dicp.ac.cn (W. Yu).

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appeared to contradict the known facts that poly-L-lysine has a larger charge density than chitosan, and a resulting stronger electrostatic interaction with alginate [4,16]. Studying the thermodynamics of interactions between oppositely charged polymers is helpful in designing novel structures of fundamental and applicative interests [20]. Though De and Robinson tried to investigate the interactions of alginate-poly-L-lysine and alginate–chitosan, the complexity of the polymeric interaction made it difficult to determine and compare the key thermodynamic parameters, such as enthalpy and stoichiometry [16]. In addition, the structure of the polycation will not only affect its interaction with alginate, but also affect the stability of polyelectrolyte complexes. The relationship between the binding enthalpy and microcapsule stability has not been established yet.

In this work, the binding properties of poly-L-lysine and chitosan on calcium alginate hydrogel beads have been evaluated quantitatively and compared, including their binding kinetics, binding capacity, binding enthalpy, and stoichiometry. The molecular state and conformation of these polycations were investigated to understand the origin of the differences in binding capacity. In addition, the properties of alginate-poly-L-lysine and alginate–chitosan complexes, including stability and mechanical strength, were measured and compared. The correlation between the stability of polyelectrolyte complexes and their thermodynamic properties was established.

2. Materials and methods

2.1. Materials components

Sodium alginate (Qingdao Crystal Salt Bioscience and Technology Corporation, Qingdao, China) with a viscosity of 100 cP at a concentration of 1% (w/v) aqueous solution at 25 °C was purchased. The compositions of the alginate molecules were characterized by ^{1}H NMR with G/M ratio of 34/66, with the molecular weight (Mw) of 430 kDa. Poly-L-lysine hydrobromide samples with Mw of 4000–15,000, 15,000–30,000, 30,000–70,000 by viscosity were from Sigma Aldrich Chemical Co (USA). The poly-L-lysine with the Mw of 15,000–30,000 was measured to be 10 kDa by gel permeation chromatography (GPC). Chitosan samples were chemically degraded from raw materials (Yuhuan Ocean Biomaterials Corporation, Zhejiang, China) by our laboratory. In brief, raw chitosan powders were dissolved in 1.0% (w/v) acetic acid, followed by adding sodium nitrite. After stirring at room temperature for 7 h, the reaction mixture was adjusted to neutral by NaOH to precipitate chitosan. The Mw of chitosan molecules were 10 kDa and 45 kDa measured by GPC technique. The degree of deacetylation (DD) was about 90% determined by Fourier transform infrared spectroscopy (FTIR).

In the following experiments, poly-L-lysine solution was made by dissolving poly-L-lysine in saline (neutral environment). In order to ensure the high charge density of chitosan, chitosan solution was made by dissolving chitosan in acetate buffer (pH = 4.3).

Fluorescein isothiocyanate (FITC, isomer I, Sigma, USA) labeled poly-L-lysine and chitosan were synthesized according to the method by Hiraku et al. [21]. All the other reagents or chemicals were all analytical-grade and used without further purification. All solutions were prepared using deionized water from Milli-Q Millipore system with a total organic carbon value of less than 15 ppb and a resistivity of 18.2 MΩ cm.

2.2. Preparation of microcapsules

The microcapsules were prepared by a two-step method. Firstly, alginate beads were formed by dropping sodium alginate solution (l.5%, w/v) into calcium chloride solution (100 mM) using an electrostatic droplet generator (YD-04, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, China). After gelling for 30 min, the beads with the diameter of 400 ± 20 μm were washed with calcium chloride solution (100 mM) for 3 times. Secondly, the beads were immersed in polycation solution with the volume of 10 times of beads to form alginate-polycation microcapsules, called alginate-poly-L-lysine (AP) and alginate–chitosan (AC) microcapsules.

2.3. Measurement of the formation kinetics of alginate-polycation microcapsules

A quantitative study of the bound polycation on the microcapsules was carried out by the measurement of concentration decrease during the binding process. Standard curves for concentration were made by ultraviolet absorption. 700 μL methyl orange was added to 100 μL polycation solutions, followed by incubation at 37 °C at the shaking speed of 150 rpm for 30 min. After that, the solution was centrifuged for 5 min, supernatant was diluted 10 times for UV measurement at 465 nm. The supernatant at certain time interval was collected and analyzed. The amount of bound polycation (mg/0.1 ml beads) was calculated as:

$$m = \frac{C_o - C_n}{Vt} \quad (1)$$

C_o and C_n are the initial polymer concentration and the polymer concentration in the supernatant after the binding process, respectively. Vt is the total volume of the solution (1.0 mL).

2.4. Isothermal titration calorimetry (ITC)

The interactions between the two different polycations and sodium alginate was probed by isothermal titration calorimetry (ITC), using a MicroCal ITC-200 instrument (Malvern Instruments Ltd.). In each measurement, nineteen injections of 0.35 wt% poly-L-lysine (27.34 mM monomer units) or 0.50 wt% chitosan (30.26 mM monomer units) were added to the sample cell filled with 0.05 wt% sodium alginate (2.53 mM monomer units) solution. The calorimeter measured the heat supplied to the sample cell to keep the cell at a constant temperature. The integration of each peak over time yielded the heat evolved (or absorbed) per mole of injected polycation. For both the two interactions, the heat effect after the saturation point was so small that the background heat of dilution was ignored. To ensure reproducibility, each ITC test was repeated three times.

2.5. Preparation of polyelectrolyte complexes

The flat polyelectrolyte complexes were prepared for fluorescence recovery after photobleaching experiments (FRAP). Alginate solution (1.5%, w/v) was cast onto a dry glass slide, then immersed in calcium chloride solution (100 mM) to form alginate hydrogels. The slides with gel were incubated in 0.1% (w/v) poly-L-lysine and chitosan solution for 10 min. The slides were subsequently washed by saline.

2.6. Circular dichroism (CD)

CD spectra were obtained at 25 °C by MOS450 (BioLogic, France). The samples were measured in a 1 mm cuvette with a volume of 400 μL. The CD signal was measured from 250 to 180 nm. The bandwidth was set to 1 nm with a response of 1 s. The secondary structure composition was calculated using the CDSSTR program, available in the CDPro software package (http://lamar.colostate.edu/ sreeram/CDPro/main.html).